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(19)

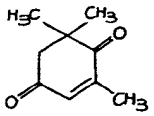
(54) A PROCESS FOR THE MANUFACTURE OF OPTICALLY ACTIVE CYCLOHEXANE DERIVATIVES

(71) We, F. HOFFMANN-LA ROCHE & CO., AKTIENGESELLSCHAFT, a Swiss Company of 124—184 Grenzacherstrasse, Basle, Switzerland, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The present invention is concerned with a process for the manufacture of optically active cyclohexane derivatives.

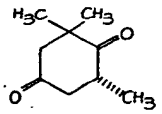
The substituents in the structural formulae given in this specification are characterised by the notation ▬ insofar as they lie in front of the plane of the molecule and by the notation |||||||| insofar as they lie behind the plane of the molecule. The substituents in the structural formulae which are not stereochemically characterised in any particular manner in this specification can have either the R or S configuration. The compounds can also be present as mixtures of the R- and S-isomers.

The process provided by the present invention for the manufacture of optically active cyclohexane derivatives comprises fermentatively hydrogenating ketoisophorone of the formula



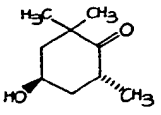
(I)

in an aqueous medium, isolating the resulting [6R]-2,2,6-trimethyl-1,4-cyclohexanedione of the formula



(II)

from the fermentation broth, subjecting said [6R]-2,2,6-trimethyl-1,4-cyclohexanedione to a reduction and, if desired, converting the resulting [4R,6R]-4-hydroxy-2,2,6-trimethylcyclohexanone of the formula



(III)

into an optically active carotenoid.

The aforementioned fermentative hydrogen can be carried out using any microorganism which is capable of converting ketoisophorone into [6R]-2,2,6-trimethyl-1,4-cyclohexanedione under aerobic or anaerobic conditions in an aqueous medium. Aerobic fermentation is preferred.

It will be appreciated that the microorganism should be cultured before use in the present fermentation; this usually being carried out in a manner known per se in an aqueous medium in the presence of the usual nutrient substances, namely in the presence of a carbon source such as glucose, fructose, saccharose and/or maltose, a nitrogen source such as urea, peptone, yeast extract, meat extract, amino acids and/or ammonium salts, inorganic salts such as magnesium, sodium, potassium, calcium and/or iron salts and other growth-promoting substances such as amino acids and vitamins. It is also often expedient to use the culture medium in the present fermentation, although, as will be more precisely described hereinafter, the composition of the fermentation medium can be substantially simpler.

The fermentation can be carried out in the absence of other additives than the ketoisophorone and the microorganism to be used. It is advantageous, however, to add a source of assimilable carbon as a microorganism nutrient substance to the aqueous medium in order to maintain the viability and the metabolic activity of the microorganism for as long as possible. The source of assimilable carbon is preferably added in an amount of 10—100 g per litre and may be, for example, a sugar such as glucose, fructose, saccharose or maltose. More than 100 g of carbon source per litre of nutrient medium does not influence the final result, but brings no other advantages than in the case when 10—100 g of carbon source are added. The addition of a nitrogen source is not necessary, but there may, however, be added a source of assimilable nitrogen, preferably in an amount of 1—50 g per litre. The source of assimilable carbon may be, for example, urea, peptone, yeast extract, meat extract, amino acids or ammonium salts. The culture medium can also contain inorganic salts such as magnesium, sodium, potassium, calcium and/or iron salts, and other growth-promoting substances such as amino acids and vitamins.

The pH at which the fermentation is carried out should preferably lie within the range from 2 to 10, especially 3—8, and this is generally achievable without special additives. If desired, the pH can be regulated by using buffers; for example phosphate, phthalate or tris buffers [tris-(hydroxymethyl)-aminomethane]. The temperature at which the fermentation is carried out can vary within wide limits (e.g. between 4°C and 50°C). A temperature of 15°C to 35°C, especially 25°C to 35°C, is preferred. In order to obtain optimal yields, it is preferred that the ketoisophorone be present in a concentration of 0.1—2.0%, especially 0.5—1.2%. After completion of the fermentative hydrogenation, fresh ketoisophorone can be added in a preferred concentration of 0.5—1%. This procedure can be repeated several times until the microorganism becomes inactivated. In a preferred fermentation procedure using press-yeast as the microorganism, there can be fermented, with periodic educt addition, up to 10%, preferably 6—8%, of ketoisophorone. The fermentation temperature in the case of this periodic educt addition is advantageously 15°—25°C.

The effective fermentation time depends on the micro-organism used, but normally varies from between 10 and 200 hours. In a preferred procedure in which the microorganism is press-yeast, the preferred fermentation time is 10 to 30 hours in a single educt addition. In the case of repeated educt addition, the fermentation time is appropriately longer and may amount to several weeks.

As mentioned earlier, the fermentation can be successfully carried out using any microorganism. The following may be mentioned as examples of representative microorganisms which can be used:

A. *Eucaryotes*

1) *Yeasts of the genera*

Candida
e.g. C. albicans
C. guillermondii
C. utilis

Kloeckera
e.g. K. brevis

Rhodotorula
e.g. R. rotundata

Saccharomyces
e.g. S. carlsbergensis
S. cerevisiae
S. cer. ellipsoides

Torula

Torulopsis
e.g. T. apicola
T. rotundata

2) *Fungi of the genera*
Aspergillus
e.g. A. clavatus
A. fischeri
A. flavus
A. fumigatus
A. ochraceus
A. wentii

Cunninghamella
e.g. C. blakesleeana

Curvularia
e.g. C lunata

Cylindrocarpon
e.g. C. radicicola

Fusarium
e.g. F. culmorum
F. solani

Hypomyces
e.g. H. rosellus

Mucor
e.g. M. circinelloides
M. corymbifer
M. griseo-cyanus
M. hiemalis
M. parasiticus
M. spinosus
M. subtilissimus

Neurospora
e.g. N. crassa

Penicillium
e.g. P. brevi-compactum
P. digitatum
P. frequentans
P. griseofulvum
P. notatum
P. novae-zeelandiae
P. viride

Rhizopus
e.g. R. arrhizus
R. nigricans
R. circinans

Trichothecium
e.g. T. roseum

B. *Procaryotes*
1) *Gram-positive bacteria of the genera*

Arthrobacter (Corynebacterium)
e.g. A. simplex (C. simplex)

Bacillus
e.g. B. megaterium
B. sphaericus
B. subtilus

Lactobacillus
e.g. L. casei rhamnosus
L. fermenti
L. leichmannii

Micrococcus
e.g. M. lysodeikticus

Propionibacterium
e.g. P. shermanii

Pediococcus
e.g. P. cerevisiae

Staphylococcus
e.g. S. albus
S. aureus

Streptococcus
e.g. S. faecalis
S. lactis

Sarcina
e.g. S. lutea

2) *Gram-negative bacteria of the genera*

Acetobacter
e.g. A. aceti
A. suboxydans

Acetomonas
e.g. A. melanogena
A. oxydans

Aerobacter
e.g. A. aerogenea

Alcaligenes
e.g. A. faecalis

Azotobacter
e.g. A. agilis
A. indicus

Escherichia
e.g. E. coli

Flavobacter
e.g. F. dehydrogenans

Klebsiella
e.g. K. pneumoniae

Pseudomonas
e.g. P. fluorescens
P. saccharophila
P. testosteroni

Proteus
e.g. P. vulgaris

Salmonella
e.g. S. typhimurium

Serratia
e.g. S. marcescens

Vibrio
e.g. V. metschnikovii

3) *Mycelium-forming bacteria (Actinomycetes) of the genera*

Actinomyces
e.g. A. cellulosae

Mycobacterium
e.g. M. butyricum
M. phlei
M. rhodochrous
M. thamnopheos

Nocardia
e.g. N. asteroides
N. brasiliensis
N. opaca

Streptomyces
e.g. S. albus (Nocardia rangoonensis)
S. fradiae
S. gelaticus
S. lavendulae
S. rimosus
S. venezuelae

Proactinomyces
e.g. P. restrictus
P. roseus

The non-specificity of the required microorganism is exemplified in that any microbially infected soil and water samples from nature are capable of being used successfully as microorganism-providers in the fermentative hydrogenation process provided by this invention.

The fermentation is generally carried out aerobically, preferably with stirring, shaking or by means of an aeration process. In order to control foam, the usual anti-foaming agents such as silicon oils, polyalkyleneglycol derivatives and soya-bean oil can be added. Having regard to the non-specificity of the required microorganism, the fermentation has the advantage that it need not be carried out under sterile conditions.

After termination of the fermentation, the [6R]-2,2,6-trimethyl-1,4-cyclohexanedione is isolated from the fermentation broth in the usual manner. Extraction with a water-insoluble organic solvent is preferably used; for example, an aliphatic or cycloaliphatic hydrocarbon which may be chlorinated such as n-hexane, cyclohexane, methylene chloride, chloroform, or carbon tetrachloride, an aliphatic ester such as ethyl acetate, n-butyl acetate or amyl acetate or an aliphatic ether such as diethyl ether or diisopropyl ether. A preferred solvent is methylene

chloride. According to a preferred isolation method, the fermented broth is filtered or centrifuged and the aqueous phase and the sediment worked up separately. The crude product obtained can be purified in the usual manner; for example by repeated recrystallisation.

The reduction of the oxo group in the 4-position of the resulting [6R]-2,2,6-trimethyl-1,4-cyclohexanedione of formula II to the hydroxy group proceeds in good yields with stereo-specific selectivity, i.e. not only with retention of the oxo function in the 1-position but also with formation of the R,R-trans configuration for the two substituents in the 4- and 6-position (hydroxy or methyl). This reduction can be carried out advantageously using an organoaluminium compound, especially a β-branched aluminium tri(lower alkyl) (e.g. triisobutylaluminium) or a corresponding halo-substituted derivative thereof (e.g. isobutylaluminium dichloride). In order to obtain optimal yields of the desired [4R,6R]-4-hydroxy-2,2,6-trimethyl-cyclohexanone of the formula III, the aluminium compound and the [6R]-2,2,6-trimethyl-1,4-cyclohexanedione of formula II should be used in approximately equimolar amounts. Other reducing agents which may be used are organic alkali metal aluminium hydrides such as sodium dihydrobis(2-methoxy-ethoxy)-aluminate and alkali metal borohydrides such as sodium borohydride. The reduction is preferably carried out in an inert organic solvent; for example, n-hexane, n-heptane, benzene, toluene, diethyl ether, tetrahydrofuran, a chlorinated hydrocarbon such as methylene chloride or chlorobenzene or mixtures of these solvents. A preferred solvent is methylene chloride and a preferred mixture consists of principally n-hexane in admixture with benzene. The reduction is preferably carried out at a temperature between about −70°C and room temperature. The reduction has the advantage that it is completed, especially when an aluminium alkyl or a halo-substituted derivative thereof is used, in a short time (generally in a few minutes at a temperature of about 0°C or above) whereafter, after neutralisation of the reduction mixture with acid, the desired [4R,6R]-4-hydroxy-2,2,6-trimethyl-cyclohexanone can be obtained by purification in the usual manner; for example, by chromatography on silica gel, aluminium oxide or dextran or by extraction using a counter-current procedure.

The stereospecific reduction in accordance with the invention can also be advantageously carried out by catalytic hydrogenation using Raney-nickel as the catalyst. This catalytic hydrogenation is preferably carried out in an inert organic solvent such as, for example, a lower alkanol such as methanol or ethanol, an ether such as diethyl ether, diisopropyl ether or tetrahydrofuran or a lower aliphatic hydrocarbon such as n-hexane. A lower alkanol such as methanol containing an approximately 5—20% addition of glacial acetic acid is preferably used. The temperature at which this catalytic hydrogenation is carried out preferably lies in a range between about 0°C and about 50°C, with room temperature being preferred. After completion of the hydrogen uptake, the mixture is separated from the catalyst and worked up in the usual manner; for example as described earlier.

The resulting [4R,6R]-4-hydroxy-2,2,6-trimethyl-cyclohexanone of formula III hereinbefore is a key substance in the manufacture of optically active carotenoids; for example, for the manufacture of:

[3R]-β-cryptoxanthin,

[3R, 3'R]-zeaxanthin,

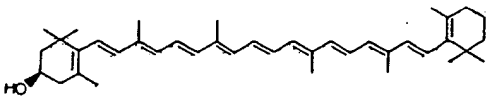
[3R]-rubixanthin,

[3R]-β-citraurin and

[3R]-reticulataxanthin.

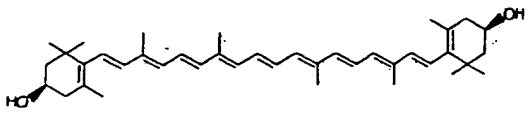
The aforementioned optically active carotenoids can be manufactured in a simple manner using methods which are known per se in carotenoid chemistry by linking a novel C_{13}, C_{15} or C_{20} building block, obtained by the chain-lengthening of [4R,6R]-4-hydroxy-2,2,6-trimethyl-cyclohexanone of formula III, with a condensation component corresponding to the desired product.

[3R]-β-cryptoxanthin of the formula



(IVa)

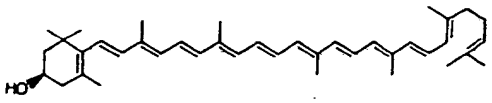
can be manufactured, for example, by condensing a [3R]-3-hydroxy-retinyl-triarylphosphonium halide with retinal and [3R, 3'R]-zeaxanthin of the formula



(IVb)

can be manufactured, for example, by condensing a[3R]-3-hydroxy-retinyl-triarylphosphonium halide with [3R]-3-hydroxy-retinal or also by condensing a 4-[[4R]-4-hydroxy-2,6,6-trimethyl-cyclohex-1-en-1-yl]-but-3-en-2-triarylphosphonium halide with 4,9-dimethyl-dodeca-2,4,6,8,10-pentaen-1,12-dial or with 4,9-dimethyl-dodeca-2,4,8,10-tetraen-6-yne-1,12-dial followed by partial hydrogenation of the resulting [3R, 3'R]-15,15'-didehydro-zeaxanthin.

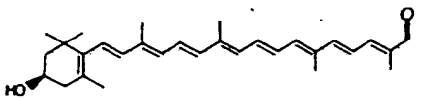
[3R]-Rubixanthin of the formula



(V)

can be manufactured, for example, by condensing a [3R]-3-hydroxy-retinyl-triarylphosphonium halide with γ-retinal.

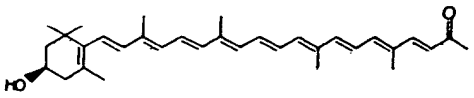
[3R]-β-citraurin of the formula



(VI)

can be manufactured, for example, by condensing a [3R]-3-hydroxy-retinyl-triarylphosphonium halide with 1,1-diethoxy-2,6-dimethyl-octa-2,4,6-trien-8-al and saponifying the acetal obtained.

[3R]-Reticulataxanthin of the formula

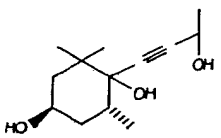


(VII)

can be manufactured, for example, by condensing [3R]-β-citraurin with acetone.

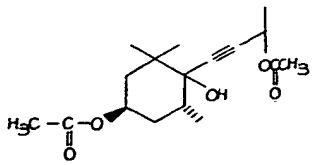
The novel C_{20} building blocks required for the syntheses outlined hereinbefore, namely the [3R]-3-hydroxy-retinyl-triarylphosphonium halides and [3R]-3-hydroxy-retinal, can be manufactured from [4R,6R]-4-hydroxy-2,2,6-trimethyl-cyclohexanone of formula III, for example by:

reacting [4R,6R]-4-hydroxy-2,2,6-trimethyl-cyclohexanone of formula III with but-3-yn-2-ol, suitably after the free hydroxy group has been masked by treatment of the ketone with isopropenylmethyl ether; acetylating the resulting 2-hydroxy-4-[[4R,6R]-1,4-dihydroxy-2,2,6-trimethyl-cyclohex-1-yl]-but-3-yne of the formula



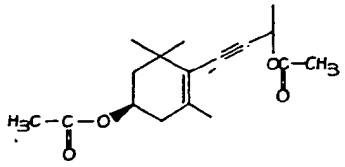
(VIII)

to give 2-acetoxy-4-[[4R,6R]-4-acetoxy-1-hydroxy-2,2,6-trimethyl-cyclohex-1-yl]-but-3-yne of the formula



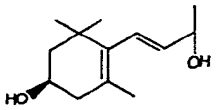
(IX);

dehydrating the diacetate of formula IX to give 2-acetoxy-4-[[4R]-4-acetoxy-2,6,6-trimethyl-cyclohex-1-en-yl]-but-3-yne of the formula



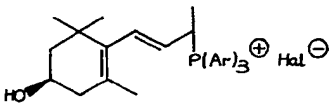
(X)

and hydrogenating the acetylenic bond present to an ethylenic bond;
converting the resulting [3R]-3-hydroxy-β-ionol of the formula



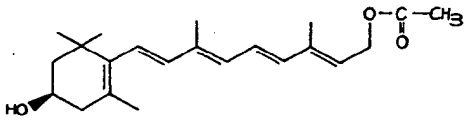
(XI)

by reaction with a triarylphosphonium halide or with a triarylphosphine in the presence of a mineral acid into a 4-[[4R]-4-hydroxy-2,6,6-trimethyl-cyclohex-1-en-1-yl]-but-3-ene-2-triarylphosphonium halide of the general formula



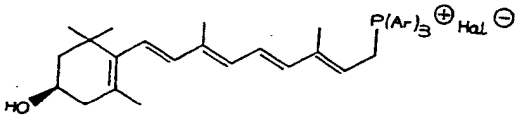
(XII),

wherein Ar represents an aryl group such as phenyl and Hal represents a halogen atom such as a bromine atom,
and condensing this Wittig salt with 1-acetoxy-3-methyl-hexa-2,4-dien-6-al to give [3R]-3-hydroxy-retinyl acetate of the formula



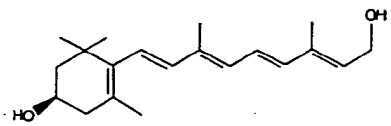
(XIII)

and
either converting said acetate of formula XIII by reaction with a triarylphosphonium halide or with a triarylphosphine in the presence of a mineral acid into a [3R]-3-hydroxy-retinyl-triarylphosphonium halide of the general formula



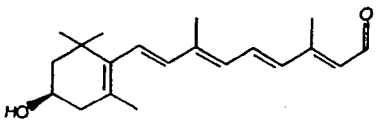
(XIV),

wherein Ar and Hal have the significance given earlier,
or saponifying said acetate of formula XIII to give [3R]-3-hydroxy-retinol of the formula



(XV)

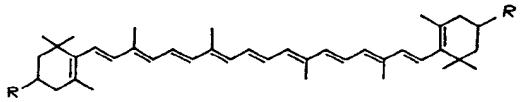
and oxidising the resulting alcohol to give [3R]-3-hydroxy-retinal of the formula



(XVI)

The C_{13} building block is the triarylphosphonium halide of formula XII hereinbefore. The C_{15} building block can be prepared, for example, according to Example 13 hereinbefore.

Of the optically active carotenoids mentioned earlier as capable of being manufactured from [4R,6R]-4-hydroxy-2,2,6-trimethyl-cyclohexanone of formula III, [3R]-β-cryptoxanthin and [3R,3'R]-zeaxanthin are preferred. Both of these, optically active carotenoids can be manufactured in the manner previously described by converting [4R,6R]-4-hydroxy-2,2,6-trimethyl-cyclohexanone of formula III, by chain lengthening procedures which are common in carotenoid chemistry, into [3R]-β-cryptoxanthin or [3R,3'R]-zeaxanthin or derivatives thereof of the general formula



(IV),

wherein the R-substituents represent hydrogen, a hydroxy group having the R-configuration or an ether or ester group convertible by hydrolysis into a hydroxy group having the R-configuration, subject to the proviso that at least one of the R-substituents represents other than hydrogen,

and hydrolysing ether or ester groups present.

The aforementioned R-substituents are, according to definition, ether or ester groups convertible by hydrolysis into hydroxy.

Ether groups convertible by hydrolysis into hydroxy are, for example, the benzyloxy group or (lower alkoxy)-(lower alkoxy) groups such as the methoxy-methoxy, α-methoxy-α-methyl-ethoxy or tetrahydropyranyloxy groups.

Ester groups convertible by hydrolysis into hydroxy are, for example, ester groups the acid part of which is derived from a lower alkanecarboxylic acid, a lower alkane-dicarboxylic acid, an aryl-(lower alkane)carboxylic acid, phosphoric acid or carbonic acid.

The esters can be manufactured in a simple manner by condensing the hydroxy compound with a corresponding acid halide (e.g. an acid chloride or bromide), a corresponding acid anhydride (e.g. acetic anhydride) or a corresponding chloroformate (e.g. trichloroethyl chloroformate).

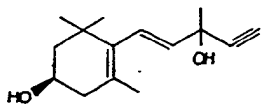
When R represents an ether group convertible by hydrolysis into hydroxy, this can be hydrolysed by treatment with a strong mineral acid (e.g. sulphuric acid or hydrochloric acid).

When R represents an ester group convertible by hydrolysis into hydroxy, this can be converted into a free hydroxy group not only by treatment with an acid but also by treatment with a base. Suitable acids are, in particular, mineral acids such as sulphuric and hydrochloric acid and suitable bases are, for example, aqueous alkali hydroxides, especially sodium hydroxide, or, preferably, alcoholic solutions of alkali hydroxides, especially alkali alcoholates such as sodium methylate.

[3R,3'R]-Zeaxanthin, which occupies a preferred position in the aforementioned optically active carotenoids, is identical with the natural carotenoid which is present, in particular, in maize. [3R,3'R]-Zeaxanthin is therefore extremely useful for the improving and colouring of foods, cosmetics and pharmaceutical preparations and is especially suitable for the pigmenting of egg-yolks and the colouring of fat and skin of poultry.

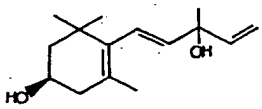
In one method according to the invention for the production of the optically active carotenoids, the [4R,6R]-4-hydroxy-2,2,6-trimethyl-cyclohexanone of formula III is reacted with but-3-yn-2-ol, the resulting 2-hydroxy-[[4R,6R]-1,4-dihydroxy-2,2,6-trimethyl-cyclohex-1-yl]-but-3-yne of formula VIII is acetylated to give 2-acetoxy-4-[[4R,6R]-4-acetoxy-1-hydroxy-2,2,6-trimethyl-cyclohex-1-yl]-but-3-yne of formula IX, the diacetate of formula IX is dehydrated to give 2-acetoxy-4-[[4R]-4-acetoxy-2,6,6-trimethyl-cyclohex-1-en-1-yl]-but-3-yne of for-

mula X and the acetylenic bond present is hydrogenated to an ethylenic bond, the resulting [3R]-3-hydroxy-β-ionol of formula XI is converted by oxidation into [3R]-3-hydroxy-β-ionone, the ionone is reacted with an alkali metal acetylide to give [3R]-3-hydroxy-ethynyl-β-ionol of formula



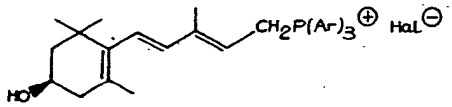
(XVII)

this latter compound is catalytically hydrogenated and the resulting [3R]-3-hydroxy-vinyl-β-ionol of formula



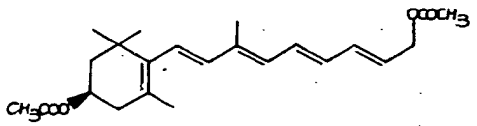
(XVIII)

is converted by reaction with a triarylphosphonium halide or with a triarylphosphine in the presence of a mineral acid into a [3R]-3-hydroxy-β-ionylidenethyl-triarylphosphonium halide of formula



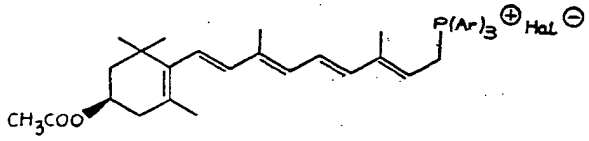
(XIX)

the phosphonium halide is reacted with γ-acetoxy-tiglic aldehyde to given [3R]-3-hydroxy-retinyl acetate of formula XIII, said acetate of formula XIII is acetylated to give [3R]-3-acetoxy-retinyl acetate of formula



(XX)

the cis form obtained is converted into trans form by isomerisation, the one half of the [3R]-3-acetoxy-retinyl acetate of formula XX is converted by reaction with a triarylphosphonium halide or with a triarylphosphine in the presence of a mineral acid into a [3R]-3-acetoxy-retinyl-triarylphosphonium halide of formula



(XXI)

and the other half of the[3R]-3-acetoxy-retinyl acetate of formula XX is saponified to give [3R]-3-hydroxy-retinol of formula XV and this alcohol is oxidised to give [3R]-3-hydroxy-retinal of formula XVI; whereafter said [3R]-3-acetoxy-retinyl-triarylphosphonium halide of formula XXI is reacted either with retinal or with [3R]-3-hydroxy-retinal of formula XVI to give [3R]-O-acetyl-β-cryptoxanthin or [3R,3'R]-O-acetyl-zeaxanthin respectively and the latter compounds are saponified to give [3R]-β-cryptoxanthin or [3R,3'R]-zeaxanthin respectively.

The compounds of formulae XIII, XV, XVI and XX are claimed in our Divisional Application No. 47891/76 (Serial No. 1,508,197). [3R,3'R]-15,15'-Didehydrozeaxanthin is claimed in our Divisional Application No. 47892/76 (Serial No. 1,508,196).

The following Examples illustrate the present invention:

Example 1.

200 litres of deionised water are sterilised in a 200 litre re-circulation fermenter together with 5 kg of household sugar and then cooled to 30°C. In this sugar solution, there are first suspended 10 kg of press-yeast (baker's yeast) and subsequently dissolved 2 kg of ketoisophorone. This batch is mixed for 36 hours at a constantly maintained temperature (30°C) with a stirrer rotation rate of 800 revolutions per minute and aerated at an air flow-rate of 3200 litres/hour. The pH value amounts to 6.6 before the beginning of fermentation and 4.6 after its termination. After 6.5 hours, there are added 20 ml of polypropyleneglycol monobutyl ether in order to control the foam. Every 3 hours, a 10 ml sample is extracted with chloroform, concentrated under reduced pressure, dried, re-dissolved in 10 ml of dioxane and analysed by gas chromatography. The percentage conversion of ketoisophorone into its dihydro derivative (the dihydro derivative obtained consists of about 95—97% of the desired [6R]-2,2,6-trimethyl-1,4-cyclohexanedione) with variation of the fermentation time is recorded in Table 1:

TABLE 1

Fermentation time in hours	% Conversion	Fermentation time in hours	% Conversion
3	9	21	70
6	19	24	74
9	32	27	79
12	45	30	80
15	55	33	82
18	63	36	82

After discontinuing the fermentation (36 hours), the fermented broth is centrifuged. Water phase and sediment are worked up separately.

The water phase (190 litres + 5 litres of wash-water) is stirred out five times with 60 litres of methylene chloride each time. The solvent phase is separated, washed twice with 60 litres of water each time and concentrated to about 15 litres on a rotary evaporator. This concentrate is dehydrated with 1.5 kg of sodium sulphate, filtered and concentrated to dryness under reduced pressure. The residue (1755 g) is dissolved while hot in 6 litres of diisopropyl ether, decolourised with 80 g of active carbon, filtered over diatomaceous earth padding and rinsed with 1.8 litres of hot diisopropyl ether. From this solution, 2.6 litres of diisopropyl ether are distilled off at normal pressure, such that the product remains dissolved in a three-fold amount of diisopropyl ether. The product is crystallised overnight at 5°C, filtered off under suction, washed twice with 1500 ml of cold n-hexane each time and dried for 15 hours at 40°C under reduced pressure. This first crystallisation produces 1280 g of optically pure [6R]-2,2,6-trimethyl-1,4-cyclohexanedione of melting point 91°—92°C. The mother liquor contains a further 447 g of substances. The latter is taken up in the same amount of n-hexane, treated with diisopropyl ether until the solution is clear, crystallised overnight at 5°C, filtered off under suction and washed twice with a small amount of cold n-hexane. The crystallisate is dried at 40°C under reduced pressure. This second crystallisation produces 83.6 g of product with a melting point of 70°—88°C. After triple recrystallisation with the three-fold amount of diisopropyl ether, there are obtained a further 43 g of optically pure [6R]-2,2,6-trimethyl-1,4-cyclohexanedione of melting point 90.5°—91.5°C.

The sediment (wet weight ca 7 kg) is stirred out twice with 70 litres of methylene chloride each time and filtered. The filtrates are washed twice with 70 litres of water each time, concentrated to 5 litres, dried with 600 g of sodium

sulphate, filtered and concentrated to dryness. The residue (82 g) is dissolved while hot in 300 ml of diisopropyl ether, decolourised with 4 g of active carbon, concentrated to 250 ml at atmospheric pressure, crystallised overnight at 5°C, filtered off under suction, washed twice with a small amount of cold n-hexane and dried at 40°C under reduced pressure. In this manner, there are obtained a further 40 g of optically pure [6R]-2,2,6-trimethyl-1,4-cyclohexanedione of melting point 90.5°—91.5°C.

The optical purity of the product is determined by NMR experiments using chiral shift reagents.

The total yield of optically pure [6R]-2,2,6-trimethyl-1,4-cyclohexanedione amounts to 1363 g. With respect to the educt (ketoisophorone) employed, this represents a relative yield of 68%.

The [6R]-2,2,6-trimethyl-1,4-cyclohexanedione shows a strongly negative Cotton effect and has a specific rotation $[\alpha]_D$ from −265° upwards (measured in methanol; c=0.4%).

Example 2.

Three soil samples taken from various places and some drops of Rhine water are individually inoculated each into 50 ml of a sterilised culture medium of the following composition:

KH_2PO_4	3.7 g/litres
Na_2HPO_4	7.0 g/litres
Yeast extract (Difco — Trade Mark)	10.0 g/litres
D(+)-Glucose monohydrate	20.0 g/litres

The batches are incubated on a shaking machine for 23 hours at a temperature of 30°C. Then, to each batch there are added a further 0.5 g of D(+)-glucose monohydrate (10 g/litres) as well as 0.05 g of ketoisophorone (1 g/litre) and the incubation is continued under the same conditions. After 7 days, a 10 ml sample is extracted with chloroform, concentrated under reduced pressure and dried. The residue is taken up in 1 ml of dioxane and analysed by gas chromatography. The percentage conversion of ketoisophorone into its dihydro derivative (the obtained dihydro derivative consists as in Example 1 predominantly of the desired [6R]-2,2,6-trimethyl-1,4-cyclohexanedione) is recorded in Table 2:

TABLE 2

Microorganisms from:	% Conversion
Soil sample 1	62.1
Soil sample 2	45.8
Soil sample 3	71.8
Rhine water	25.2

Example 3.

Two conversion Experiments A and B are carried out in clean but not sterilised small fermenters using ketoisophorone as the substrate. The fermenters are each charged with the following substances:

TABLE 3

Substance	Experiment A	Experiment B
De-ionised water (not sterile)	4000 ml	3920 ml
Crystalline sugar	80 g	40 g
Press-yeast	80 g	80 g
Ketoisophorone	40 g	48 g

The conversions are carried out under the following conditions:

Experiment A:

Temperature:	30°C
Aeration:	Surface aeration, i.e. the air supply is introduced into the gas space above the broth. Air flow 240 litres/hours.
Stirrer rate:	1000 revolutions per minute
pH:	3.8—3.9
Fermentation time:	77 hours

Experiment B:

Temperature:	30°C
Aeration:	Through-flow aeration, i.e. the air supply is introduced into the broth below the stirring propellor. Air flow about 10 litres/hours.
Stirrer rate:	1000 revolutions per minute
pH:	3.6—4.0
Fermentation time:	142 hours

After a fermentation time of 48 hours, a further 40 g of sugar and 80 g of press-yeast are added in Experiment B.

The progress of the fermentations A and B is monitored by gas chromatographic analysis of the chloroform extracts from 5 ml samples. The percentage conversion of ketoisophorone into its dihydro derivative amounts to 84% in Experiment A and 82% in Experiment B (the dihydro derivative obtained consisting as in Example 1 predominantly of the desired [6R]-2,2,6-trimethyl-1,4-cyclohexanedione).

In both batches, the fermentation product is isolated as follows:

The unfiltered broth is extracted twice with the threefold volume of methylene chloride. The organic phase is dried over sodium sulphate and concentrated under reduced pressure. The crystalline crude product is dissolved in the five-fold volume of benzene, percolated over the three-fold amount of silica gel and again concentrated under reduced pressure. The colourless residue is dissolved while hot in the five-fold volume of n-hexane and crystallised overnight at room temperature. After removal of the solvent by suction and drying the

crystals under reduced pressure at 40°C, there is obtained optically pure [6R]-2,2,6-trimethyl-1,4-cyclohexanedione.

The optical purity of the product is determined by NMR experiments using chiral shift reagents.

From Experiment A there are isolated 17.5 g and from Experiment B 27.8 g of pure [6R]-2,2,6-trimethyl-1,4-cyclohexanedione with a melting point of 90°—92°C. Accordingly, the relative net yields (product amount with respect to the educt employed) amounts to 43% and 58% respectively.

Example 4.

The conversion of ketoisophorone with semi-continuous educt addition is carried out at 20°C both in a laboratory fermenter (working volume: 5 litres) and in a large re-circulation fermenter (working volume: 160 litres). Without sterilisation, the two fermenters are charged respectively with 4.75 litres and 150 litres of deionised water in which are suspended respectively 250 g and 8 kg of press-yeast. The 5 litre fermenter is aerated by introducing an air stream of 360 litres/hour into the gas space above the broth and mixed with a baffle stirrer set at 1100 revolutions per minute. In the 160 litre fermenter, an air stream of 3200 litres/hour is introduced into the fermentation broth and the latter is mixed with the re-circulation system set at a stirring rate of 800 revolutions per minute. Ketoisophorone and sugar are added as set out in Table 4:

TABLE 4

5 Litre fermenter			160 Litre fermenter		
Time (hours)	Ketoisophorone	Sugar	Time (hours)	Ketoisophorone	Sugar
0	50 g	125 g	0	1.6 kg	4.0 kg
48	50 g		46	0.8 kg	0.8 kg
78	25 g		70	0.8 kg	0.8 kg
94	25 g	50 g	94	0.8 kg	
102	25 g		118	0.8 kg	
126	25 g		146	0.8 kg	1.6 kg
149	25 g		170	0.8 kg	
168	25 g	50 g	194	0.8 kg	
192	25 g		218	0.8 kg	1.6 kg
216	25 g		242	0.8 kg	
243	25 g		286	0.8 kg	
267	25 g	50 g	310		1.6 kg
291	25 g				
335	25 g				
358		50 g			
Total weight	400 g	325 g	Total weight	9.6 kg	10.4 kg

In the 5 litre fermenter there is accordingly used a total of 80 g/litre of ketoisophorone with a sugar consumption of 65 g/litre and fermentation time of 17 days (406 hours). In the 160 litre fermenter there are used 60 g/litre of ketoisophorone with a sugar consumption of 65 g/litre and fermentation time of 16 days (384 hours). After 126 hours of fermentation time, 16 ml of propyleneglycol monobutyl ether are added in order to control the foam. The progress of the conversion reactions is monitored by regular gas chromatographic analysis of the concentrated chloroform extracts from 5 ml samples. The dihydro derivative begins to crystallise out at concentrations above 10 g/litre. After termination of the fermentation, the chloroform extracts from the 5 litre and 160 litre fermenters contain respectively 93% and 87% of the desired dihydro derivative.

The product from the 5 litre fermenter is isolated as follows:

The fermentation broth is cooled to 11°C and the resulting crystals separated using a coarse filter. A portion of the mycelium is also retained on the filter. The residue and the filtrate are extracted twice with the three-fold amount of methylene chloride. The organic phase is dried over sodium sulphate and concentrated under reduced pressure. The crystalline crude extract is recrystallised from diisopropyl ether. From the filter residue there are isolated 257.3 g of optically pure product of melting point 91°—93°C. From the filtrate and from the mother liquor there are obtained respectively a further 30.5 g and a further 15.3 g of optically pure product of melting point 91°—93°C. The optical purity can be determined by NMR examination using Eu (HFC)$_3$ as the shift reagent and by measuring the optical rotation. The total yield of [6R]-2,2,6-trimethyl-1,4-cyclohexanedione amounts to 303.1 g (75.8% yield with respect to the educt employed). (Eu(HFC)$_3$ is tris[3-(heptafluoropropylhydroxymethylene)-d-camphorato]-europium).

The product from the 160 litre fermenter is isolated as follows:

The broth is cooled to ca 10°C, mixed with 5 kg of diatomaceous earth and then centrifuged. The fermenter is rinsed with 20 litres of water. The sediment (crystalline product and mycelium) is extracted four times with 50 litres of methylene chloride each time. The organic phase is subsequently used as an extractant for the supernatant [first extraction: 100 litres; second and third extractions: each 50 litres], separated, washed twice with 30 litres of water each time, concentrated to ca 20 litres on a rotary evaporator, dried over sodium sulphate and concentrated to constant weight under reduced pressure. The crystalline crude extract obtained in this manner is dissolved while hot in 24 litres of diisopropyl ether, decolourised with 200 g of active carbon, filtered over diatomaceous earth padding and recrystallised overnight at 5°C. The crystallisate is filtered off under suction, washed twice with 10 litres of cold hexane (0°C) each time and dried under reduced pressure at 30°C. In this manner there are obtained 6250 g of optically pure [6R]-2,2,6-trimethyl-1,4-cyclohexanedione of melting point 91°—92°C. The mother liquor is concentrated to a volume of ca 3 litres and the substance contained therein is crystallised out overnight at 5°C. The crystallisate is in turn filtered off under suction, washed twice with 500 ml of cold hexane and dried under reduced pressure at 35°C. There are obtained a further 442 g of product with a melting point of 88°—89°C. The product is again recrystallised overnight at 5°C from 1.3 litres of diisopropyl ether, washed twice with 500 ml of cold hexane each time and dried under reduced pressure at 35°C. There thus result 399 g of optically pure [6R]-2,2,6-trimethyl-1,4-cyclohexanedione of melting point 90.5°—91.5°C. The total yield of optically pure product amounts to 6649 g (69.3% yield with respect to the substrate employed). The optical purity is again determined by NMR examination using Eu (HFC)$_3$ as the shift reagent.

Example 5.

The ability to convert ketoisophorone into its dihydro derivative is tested in 99 different microorganisms which are selected from the following groups:

A) *Eucaryotes*
1) *Yeasts of the genera:*
Candida
Kloeckera
Rhodotorula
Saccharomyces
Torula
Torulopsis

2) *Fungi of the genera:*
Aspergillus
Cunninghamella
Curvularia
Cylindrocarpon
Fusarium
Hypomyces
Mucor
Neurospora
Penicillium
Rhizopus
Trichothecium

B) *Procaryotes*
1) *Gram-positive bacteria of the genera:*
Arthrobacter (Corynebacterium)
Bacillus
Lactobacillus
Micrococcus
Propionibacterium
Pediococcus
Staphylococcus
Streptococcus
Sarcina

2) *Gram-negative bacteria of the genera:*
Acetobacter
Acetomonas
Aerobacter
Alcaligenes
Azobacter
Escherichia
Flavobacter
Klebsiella
Pseudomonas
Proteus
Salmonella
Serratia
Vibrio

3) *Mycelium-forming bacteria (Actinomycetes) of the genera:*
Actinomyces
Mycobacterium
Nocardia
Streptomyces
Proactinomyces

Using customary microbiological techniques, the microorganisms are inoculated in 50 ml of a complex culture medium and incubated for 48—72 hours on a shaking machine at 30°C. The medium is composed as follows:

KH_2PO_4	3.7 g/litres
Na_2HPO_4	7.0 g/litres
Yeast extract (Difco)	10.0 g/litres
D (+)-Glucose monohydrate	20.0 g/litres

After 48—72 hours incubation time, a further 0.5 g of D (+)-glucose monohydrate (10 g/litres) as well as 0.05 g of ketoisophorone (1 g/litre) are added to each 50 ml batch and the incubation is continued under the same conditions for 1 week. After 1 day and after 7 days, each 10 ml of cell suspension from all batches is extracted twice with chloroform, the organic phase obtained is concentrated under reduced pressure at 40°C and dried. The residue is taken up in 1 ml of

dioxane and analysed by gas chromatography. As will be seen from Table 5, all of the microorganisms are capable of converting ketoisophorone into its dihydro derivative. The dihydro derivative obtained consists as in Example 1 predominantly of the desired [6R]-2,2,6-trimethyl-1,4-cyclohexanedione, which can be isolated in the same manner as given in Example 1 or 4. In Table 5,

\+ signifies 0.1—10% conversion
++ signifies 10.1—30% conversion
+++ signifies 30.1—50% conversion
++++ signifies 50.1—70% conversion
+++++ signifies over 70% conversion

of ketoisophorone into its dihydro derivative:

TABLE 5

Number	YEASTS Microorganism	Conversion after 1 day	7 days
1	Candida albicans	+++	+
2	,, guillermondii	+++	+
3	,, utilis	++	+++++
4	Kloeckera brevis strain 1	+++++	+++++
5	,, brevis strain 2	+++++	+++++
6	Rhodotorula sp.	+++	+
7	,, rotundata	++	+
8	Saccharomyces carlsbergensis	++++	+++++
9	,, cerevisiae strain 1	++++	++++
10	,, ,, strain 2	++	+
11	,, ,, strain 3	++	++
12	,, cer. ellipsoides	++++	++++
13	Torula sp.	+++	+
14	Torulopsis apicola	+	+
15	,, rotundata	+++	—

TABLE 5 (Continued)

Number	FUNGI Microorganism	Conversion after 1 day	7 days
16	Aspergillus clavatus	+++++	++
17	,, fischeri	+++	+++
18	,, flavus	++++	++++
19	,, fumigatus Fres.	++	+
20	,, ochraceus	++	++++
21	,, sp.	++	+
22	,, wentii Wehmer	+++	++++
23	Cunninghamella blakesleena (Lendner)	++	++
24	Curvularia lunata (Wakker Boedijn)	+	+++
25	Cylindrocarpon radicicola	+++	+++
26	Fusarium culmorum	+++++	++++
27	,, solani	++	++++
28	Hypomyces rosellus (Dactylium dendroides)	+	++
29	Mucor circinelloides	++++	++
30	,, corymbifer (Absidia lichtheimi)	+++	++++
31	,, griseo-cyanus	++++	+
32	,, hiemalis Wehmer	++++	++
33	,, parasiticus	++++	+++
34	,, spinosus	++	+
35	,, subtilissimus	++++	+
36	Neurospora crassa	++++	+++
37	Penicillium brevi-compactum	+++++	++
38	,, digitatum	+	+
39	,, frequentans	+++	+++
40	,, griseofulvum	+	++
41	,, notatum	++	+
42	,, novae-zeelandiae	++++	++
43	,, viride	++	+

TABLE 5 (Continued)

Number	FUNGI (Continued) Microorganism	Conversion after 1 day	7 days
44	Rhizopus arrhizus	+++++	+++
45	,, nigricans Ehrenberg	+++++	+++++
46	,, circinans (Rhizopus reflexus Bain)	++++	+++
47	,, circinans v. Tiegheim	++++	+
48	Trichothecium roseum	+	+

TABLE 5 (Continued)

Number	Gram-positive BACTERIA Microorganism	Conversion after 1 day	7 days
49	Arthrobacter simplex (Corynebact. simpl.)	+	++
50	Bacillus megaterium	++	++++
51	,, sphaericus	+	++
52	,, subtilis	+++	+++++
53	Lactobacillus casei rhamnosus	+	+
54	,, fermenti	+	+
55	,, leichmannii	+	++
56	Micrococcus lysodeikticus	+	+++++
57	Propionibacterium shermanii	++	+++++
58	Pediococcus cerevisiae	+	+
59	Staphylococcus albus	+	+
60	,, aureus	+	+
61	Streptococcus faecalis	+	+
62	,, lactis	+	+
63	Sarcina lutea	+	++

TABLE 5 (Continued)

Number	Gram-negative BACTERIA Microorganism	Conversion after 1 day	Conversion after 7 days
64	Acetobacter aceti	++	++
65	,, suboxydans strain 1	++++	++++
66	,, ,, strain 2	++	++
67	Acetomonas melanogena	+	++
68	,, oxydans	++++	++++
69	Aerobacter aerogenea	++	++++
70	Alcaligenes faecalis	++	++++
71	Azotobacter agilis	++	++++
72	,, indicus	++	++++
73	Escherichia coli	++	++
74	Flavobacter dehydrogenans	+	+
75	Klebsiella pneumoniae	++	++++
76	Pseudomonas fluorescens	+	+++
77	,, saccharophila	++	++
78	,, testosteroni	++	++++
79	Proteus vulgaris	+++	+++++
80	Salmonella typhimurium	++	++++
81	Serratia marcescens	++	+++
82	Vibrio metschnikovii	++	++++

TABLE 5 (Continued)

Number	ACTINOMYCETES (mycelium-forming bacteria) Microorganism	Conversion after 1 day	7 days
83	Actinomyces cellulosae	+	++
84	Mycobacterium butyricum	+++	+++
85	,, phlei	++	+++
86	,, ,,	++	++
87	,, rhodochrous	+++	+++
88	,, thamnopheos	+	+
89	Nocardia asteroides	++++	+
90	,, brasiliensis	++	+++++
91	,, opaca	++	++
92	Streptomyces albus (Nocardia rangoonensis)	++	+++
93	,, fradiae	+	+
94	,, gelaticus Waksman	++	+++
95	,, lavendulae	++	++
96	,, rimosus	++	+
97	,, venezuelae	+	+
98	Proactinomyces restrictus Turfitt (Noc. rest.)	+	+
99	,, roseus	++	++

Example 6.

A solution of 20 g (130 mmol) of [6R]-2,2,6-trimethyl-1,4-cyclohexanedione in 1550 ml of a mixture of n-hexane and benzene (volumetric proportion 7:3) is cooled in an argon atmosphere to −5°C in a four-necked flask provided with thermometer, stirrer, gassing fitment and calcium chloride tube. The gassing fitment is removed and replaced by a dropping funnel. The cooled solution is treated within about 4 minutes while stirring vigorously, with 173 ml of a 0.81—M solution of triisobutylaluminium in toluene (140 mmol) via the dropping funnel in such a manner that the internal temperature is maintained between −4°C and 0°C. The mixture is then mixed with 1085 ml of 5% aqueous hydrochloric acid. Both phases are separated from one another after about 30 minutes and the aqueous phase is extracted with methylene chloride. The combined organic phases are washed to neutrality with water, dried over sodium sulphate and evaporated under reduced pressure. There are obtained 18.7 g of a yellow oil which, according to the gas chromatogram, consists of 63% of trans-4-hydroxy-6-methyl compound. After chromatographic purification of this oil on silica gel (0.06—0.2 mm) using n-hexane/ether (80/20) as the eluant, there are obtained 11.6 g of a product which, after recrystallisation twice from n-hexane/diisopropyl ether at −70°C, yields 10.0 g (50%) of [4R,6R]-4-hydroxy-2,2,6-trimethyl-cyclohexanone as colourless crystals of melting point 49°—50°C.

The optical purity of the product is determined by NMR examination using chiral shift reagents.

Isobutylaluminium dichloride can be used instead of the triisobutylaluminium to give similar results.

Example 7.

A suspension of 30 g of Raney nickel in 200 ml of methanol in a round-bottomed flask is treated with 65 ml of glacial acetic acid while stirring. After the addition of 10 g (65 mmol) of [6R]-2,2,6-trimethyl-1,4-cyclohexanedione in 300 ml of methanol, hydrogen gas is introduced into the mixture while vigorously shaking at room temperature. After hydrogenation for 13 hours (hydrogen uptake 955 ml), the reaction product is separated from the catalyst, neutralised with sodium bicarbonate and extracted with methylene chloride. There is obtained a yellow oil which, according to the gas chromatogram, consists of 81% of trans-4-hydroxy-6-methyl compound. According to NMR examination using chiral shift reagents, the trans compound consists of 67% of [4R,6R]-4-hydroxy-2,2,6-trimethyl-cyclohexanone. The oil is worked up in the same manner as given in Example 8. There is obtained [4R,6R]-4-hydroxy-2,2,6-trimethyl-cyclohexanone, which is identical with the compound obtained according to Example 8.

Example 8.

A suspension of 30 g of Raney nickel in 150 ml of ether in a round-bottomed flask is treated with 10 g (65 mmol) of [6R]-2,2,6-trimethyl-1,4-cyclohexanedione in 150 ml of ether. Hydrogen gas is now introduced into the mixture while vigorously shaking at room temperature. After hydrogenation for 45 minutes (hydrogen uptake 1160 ml), the reaction product is separated from the catalyst and the catalyst is washed with 200 ml of ether. The ether phase is evaporated under reduced pressure. There are obtained 10 g of a yellow oil which, according to the gas chromatogram, consists of 63% of trans-4-hydroxy-6-methyl compound. After chromatographic purification of this oil on silica gel (0.06—0.2 mm) with n-hexane/ether (80/20) as the eluant, there are obtained 4.65 g of a product which, after recrystallisation twice from n-hexane/diisopropyl ether at −70°C, yields 3.0 g (30%) of [4R,6R]-4-hydroxy-2,2,6-trimethyl-cyclohexanone as colourless crystals of melting point 49°—50°C.

The optical purity of the product is determined by NMR examination using chiral shift reagents.

Example 9.

A solution of 120 g (778 mmol) of [6R]-2,2,6-trimethyl-1,4-cyclohexanedione in 4680 ml of toluene is cooled to −40°C under an argon atmosphere in a 10 litre sulphonating flask provided with thermometer, stirrer, gassing attachment and calcium chloride tube. The suspension resulting from partial crystallisation is now treated within less than 20 seconds while continuously stirring and with the cooling bath still in place, with 1080 ml of a 20% solution of triisobutylaluminium in toluene (1090 mmol). The internal temperature, which rises to ca 22°C during this addition, is immediately lowered to −40°C again (about 4 minutes) by the constant cooling. The mixture is left for a further 80 minutes at −40±2°C and then treated within 30 seconds with 1344 ml of 10% hydrochloric acid (4160 mmol). The two-phase mixture is stirred for a further 30 minutes without cooling and then rinsed into a 15 litre stirring vessel. The mixture is extracted in three extraction stages with a total of 2800 ml of methylene chloride. The organic phases are washed to neutrality with water, combined, dried over sodium sulphate and evaporated under reduced pressure. There are obtained 119.2 g of a yellow oil which, according to the gas chromatogram, consists of 66.7% of trans-4-hydroxy-6-methyl compound [18% of starting material remain in unchanged form and can be recycled depending on the isolation/purification process]. After chromatographic purification of this oil on silica gel (0.06—0.2 mm) using n-hexane/ether (70:30) as the eluant, there are obtained 79 g of product. Double recrystallisation from n-hexane/diisopropyl ether at −45°C yields 64 g (53%) of [4R,6R]-4-hydroxy-2,2,6-trimethyl-cyclohexanone as colourless crystals of melting point 49°—50°C.

Example 10.

9.8 g of [4R,6R]-4-hydroxy-2,2,6-trimethyl-cyclohexanone are dissolved in 6.8 g of isopropenylmethyl ether. The solution is treated in the cold with 4 drops of a 1% methanolic solution of p-toluenesulphonic acid, then neutralised by the addition of triethylamine and subsequently evaporated under reduced pressure. The resulting [4R,4'R]-4,4'-(isopropylidenedioxy)-bis[[6R]-2,2,6-trimethylcyclohexanone] melts at 109°—111°C after recrystallisation from hexane.

A solution of ethylmagnesium bromide in tetrahydrofuran (prepared in the usual manner from 18.2 g of magnesium, 81.8 g of ethyl bromide and 200 ml of tetrahydrofuran) is treated dropwise within 30 minutes at room temperature, with 26.6 g of but-3-yn-2-ol in 75 ml of tetrahydrofuran. The mixture is stirred under reflux conditions of 2 hours and subsequently treated dropwise with a solution of 11.1 g of [4R,4'R]-4,4'-(isopropylidenedioxy)-bis[[6R]-2,2,6-trimethylcyclohexanone] in 75 ml of tetrahydrofuran. The mixture is stirred for 12 hours under reflux conditions, subsequently acidified by the addition of 1-N sulphuric acid, then saturated with common salt and extracted with ether. The ether extract is washed to neutrality with an aqueous common salt solution, dried over sodium sulphate and evaporated under reduced pressure. The resulting oily 4-[[4R,6R]-1,4-dihydroxy-2,2,6-trimethylcyclohex-1-yl]-but-3-yn-2-ol is subsequently acetylated by treatment with acetic anhydride in the presence of pyridine. There is obtained 2-acetoxy-4-[[4R,6R]-1-hydroxy-4-acetoxy-2,2,6-trimethyl-cyclohex-1-yl]-but-3-yne as an oil, which is purified by adsorption on silica gel using n-hexane/ether (3:2) as the eluant.

8.6 g of 2-acetoxy-4-[[4R,6R]-1-hydroxy-4-acetoxy-2,2,6-trimethyl-cyclohex-1-yl]-but-3-yne are dissolved in a mixture of 53.5 ml of pyridine and 22 ml of phosphorus oxychloride and heated to 100°C for 18 hours. The mixture is then cooled and introduced into ice/water. The mixture is extracted with ether and the ether extract is washed to neutrality with water and 1-N sulphuric acid, dried over sodium sulphate and evaporated under reduced pressure. The resulting oily 2-acetoxy-4-[[4R]-4-acetoxy-2,6,6-trimethylcyclohex-1-en-1-yl]-but-3-yne is purified by adsorption on silica gel using hexane/ether (4:1) as the eluant.

4.0 g of 2-acetoxy-4-[[4R]-4-acetoxy-2,6,6-trimethylcyclohex-1-en-1-yl]-but-3-yne are dissolved in 50 ml of absolute tetrahydrofuran. The solution is added dropwise to a suspension of 2.4 g of lithiumaluminium hydride in 180 ml of tetrahydrofuran while stirring at room temperature and the mixture obtained is heated under reflux conditions for 12 hours. The mixture is cooled, treated successively with aqueous ether and an aqueous ammonium chloride solution, then saturated with common salt and thoroughly extracted with ether. The ether extract is washed to neutrality, dried and evaporated. The resulting oily 4-[[4R]-4-hydroxy-2,6,6-trimethyl-cyclohex-1-en-1-yl]-but-3-en-2-ol [[3R]-3-hydroxy-β-ionol] is purified by adsorption on silica gel using hexane/ether (1:1) as the eluant.

2.1 g of [3R]-3-hydroxy-β-ionol are dissolved in 50 ml of absolute methanol. After the addition of 3.43 g of triphenylphosphine hydrobromide, the solution is stirred at room temperature for 12 hours. The solvent is subsequently evaporated under reduced pressure. The residue is dissolved in 80% aqueous isopropanol and shaken out twice with hexane. The isopropanol phase is evaporated under reduced pressure. The residue is dissolved in methylene chloride, dried over sodium sulphate and evaporated under reduced pressure. The remaining 4-[[4R]-4-hydroxy-2,6,6-trimethyl-cyclohex-1-en-yl]-3-en-2-triphenylphosphonium bromide is further reacted as follows:

16.05 g of 4-[[4R]-4-hydroxy-2,6,6-trimethyl-cyclohexyl-en-1-yl]-but-3-ene-2-triphenylphosphonium bromide and 5.39 g of 6-acetoxy-4-methyl-hexa-2,4-dien-1-al are dissolved in 100 ml of isopropanol. The solution is treated dropwise while stirring at −35°C with a solution of 2.09 g of 86% potassium hydroxide in 1.5 ml of water. In so doing, the internal temperature rises to −20°C. The mixture is subsequently diluted with 100 ml of cold low-boiling petroleum ether and introduced into a mixture of 100 ml of low-boiling petroleum ether and 100 ml of ice/water. The petroleum ether phase which separates is thoroughly washed with a total of 120 ml of methanol/water (80:20), then dried over sodium sulphate and evaporated under reduced pressure. The resulting [3R]-3-hydroxy-retinyl acetate, which consists of about 73% 9-cis- and about 27% all-trans-[3R]-3-hydroxy-retinyl acetate, can be isomerised, for example, according to one of the following methods a) or b):

a) 3 g of the 9-cis/all-trans-[3R]-3-hydroxy-retinyl acetate isomer mixture are dissolved in 15 ml of acetonitrile. After the addition of 6 g of palladium oxide/barium sulphate catalyst (the carrier contains 0.5% palladium), the mixture is heated at 70°C for 1 hour while stirring. After cooling, the catalyst is filtered off and the filtrate evaporated in vacuo. The resulting isomer mixture consists of about 74% all-trans- and about 26% 9-cis-[3R]-3-hydroxy-retinyl acetate.

b) 3.2 g of the 9-cis/all-trans-[3R]-3-hydroxy-retinyl acetate isomer mixture are dissolved in 6.5 ml of acetonitrile. After the addition of 30 mg of $Pd(C_6H_7CN)_2Cl_2$ and 0.03 ml of triethylamine, the mixture is stirred at 65°C for 1 hour. After

cooling, the mixture is diluted with 10 ml of water and extracted with ether. The ether extract is washed with water, dried and evaporated. The resulting isomer mixture consists of about 78% all-trans- and about 22% 9-cis-[3R]-3-hydroxy-retinyl acetate.

The isomer mixture obtained according to method a) or b) can be separated further by crystallisation in the normal manner in order to increase the all-trans portion.

The [3R]-3-hydroxy-retinyl acetate prepared in the foregoing can be used in the manufacture of [3R,3'R]-zeaxanthin; for example according to the following method:

5 g of [3R]-3-hydroxy-retinyl acetate are dissolved in 16.5 ml of ethanol. The solution is treated dropwise at 40°C within 15 minutes with a solution of about 1.85 g of sodium hydroxide in 7.5 ml of water. The mixture is stirred for 30 minutes at 40°C, then cooled to 10°C and extracted with 20 ml of low-boiling petroleum ether. The extract is washed to neutrality with ice/water, dried and evaporated. There is obtained [3R]-3-hydroxy-retinol. 5 g of [3R]-3-hydroxy-retinol are dissolved in 50 ml of methylene chloride. After the addition of 30 g of manganese dioxide, the solution is stirred at room temperature for 24 hours. The unconsumed manganese dioxide is filtered off and rinsed with 30 ml of methylene chloride. The washings are combined with the filtrate and evaporated under reduced pressure. The residue is dissolved in 15 ml of low-boiling petroleum ether with warming. The solution is slowly cooled to −40°C. The precipitated [3R]-3-hydroxy-retinal is filtered off, washed with cold petroleum ether and dried in vacuo at room temperature. The aldehyde can be condensed without further purification with [3R]-3-hydroxy-retinyl-triphenylphosphonium bromide to give [3R,3'R]-zeaxanthin:

3.78 g of [3R]-3-hydroxy-retinyl acetate are dissolved in 10 ml of absolute methanol. After the addition of 4.15 g of triphenylphosphine hydrobromide, the solution is stirred for 12 hours at room temperature. The resulting solution of [3R]-3-hydroxy-retinyl-triphenylphosphonium bromide is diluted with 50 ml of chloroform. The solution is treated dropwise at 0°—5°C simultaneously with a solution of 0.55 g of sodium in 5.5 ml of methanol and a solution of 3.0 g of [3R]-3-hydroxy-retinal in 10 ml of chloroform. The mixture is subsequently stirred for 1 hour at room temperature, then treated with 0.57 ml of glacial acetic acid and washed twice with 50 ml each time of a 5% aqueous sodium bicarbonate solution. The washings are shaken out twice with 10 ml of chloroform each time. The chloroform extracts are combined with the original chloroform solution, dried over sodium sulphate and evaporated under reduced pressure, the chloroform being successively replaced by methanol. The solvent is subsequently evaporated down to ca 50 ml. After the addition of 2.5 ml of water, the concentrate is cooled to −20°C. The precipitated [3R,3'R]-zeaxanthin is recrystallised from methylene chloride/pentane; melting point 201°—203°C.

Example 11.

1.605 g of 4-[[4R]-4-hydroxy-2,6,6-trimethyl-cyclohex-1-en-1-yl]-but-3-ene-2-triphenylphosphonium bromide obtained according to Example 10 are dissolved in 10 ml of isopropanol and introduced at room temperature into a solution of 214 mg of 4,9-dimethyl-dodeca-2,4,8,10-tetraen-6-yne-1,12-dial [C_{14} aldehyde] in 10 ml of methylene chloride while stirring. The resulting homogeneous solution is treated with 0.336 ml of a 50% aqueous potassium hydroxide solution. The initially weakly yellow solution turns dark red after 2 to 3 minutes. The solution is stirred for a further 90 minutes at room temperature, then thoroughly extracted with methylene chloride. The combined methylene chloride extracts are washed to neutrality with water, dried over sodium sulphate and evaporated under reduced pressure. There is obtained crude cis/trans-[3R,3'R]-15,15'-didehydro-zeaxanthin which is brought to crystallisation by trituration with 3 ml of methanol in the cold, filtered off, dried and then subjected to the following isomerisation:

468 mg of cis/trans; [3R,3'R]-15,15'-didehydro-zeaxanthin are dissolved in 18 ml of acetonitrile. The solution is treated with 936 mg of a palladium oxide/barium sulphate catalyst containing 0.5% palladium, stirred for 12 hours at 70°C and subsequently cooled to room temperature. The catalyst is separated and repeatedly washed with a total of 60 ml of methylene chloride. The washings are combined with the filtrate and evaporated under reduced pressure. There is obtained crystalline all-trans-[3R,3'R]-15,15'-didehydro-zeaxanthin which melts at 208°—210°C after recrystallisation from methylene chloride and hexane.

426 mg of palladium/calcium carbonate partially inactivated catalyst are suspended in 34 ml of absolute toluene and, after the addition of 46 ml of absolute ethyl acetate and 0.0125 ml of quinoline, pre-hydrogenated. After termination of the hydrogen uptake, the catalyst mixture is treated with 213 mg of all-trans-[3R,3'R]-15,15'-didehydro-zeaxanthin and hydrogenated further at atmospheric pressure and room temperature until uptake of 8.43 ml of hydrogen. The catalyst is filtered off and washed with ethyl acetate. The washings are combined with the filtrate, washed 3 times with 2 ml of 0.1-N sulphuric acid each time and then with water, dried over sodium sulphate and evaporated under reduced pressure. There is obtained partly oily [3R,3'R]-15-cis-zeaxanthin which is suspended in 15 ml of heptane and isomerised at 100°—110°C for 3.5 hours. All-trans-[3R,3'R]-zeaxanthin is precipitated crystalline in the cold; melting point 208.5°—209.5°C after recrystallisation from methylene chloride/methanol.

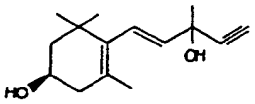
Example 12.

If in the procedure described in Example 11, the 4,9-dimethyl-dodeca-2,4,8,10-tetraen-6-yne-1,12-dial is replaced by 4,9-dimethyl-dodeca-2,4,6,8,10-pentaene-1,2,-dial, then after condensation with 4-[[4R]-4-hydroxy-2,6,6-trimethylcyclohex-1-en-1-yl]-but-3-ene-2-triphenylphosphonium bromide and subsequent isomerisation of the resulting cis/trans-[3R,3'R]-zeaxanthin, there is obtained directly all-trans-[3R,3'R]-zeaxanthin; melting point 208°—209°C after recrystalisation from methylene chloride/methanol.

Example 13.

20 g of [3R]-3-hydroxy-β-ionol and 30 g of 2,3-dichloro-5,6-dicyano-benzoquinone are dissolved in 400 ml of absolute dioxane. The solvent is heated for 1.5 hours at 50°—55°C. The solution is subsequently cooled to 0°C and the precipitated 2,3-dichloro-5,6-cyano-benzohydroquinone is filtered off. The filtrate is evaporated at 50°C under reduced pressure. The residue is dissolved in 250 ml of ether and extracted with a solution of 50 g of sodium dithionite in 250 ml of water. The ethereal phase is subsequently washed neutral with saturated aqueous sodium chloride solution, 1—N aqueous sodium hydroxide solution and again with saturated aqueous sodium chloride solution, dried over sodium sulphate and evaporated to dryness. The residual [3R]-3-hydroxy-β-ionone can be purified by adsorptionon silica gel (elution with ether) and is further reacted as follows:

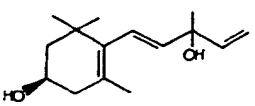
To a solution of sodium acetylide in liquid ammonia (prepared from 60 ml of liquid ammonia, 2.68 g of sodium and acetylene in the usual manner) there are first added 6.0 ml of absolute ether and then, dropwise with stirring, a solution of 6.65 g of [3R]-3-hydroxy-β-ionone in 12 ml of ether. The mixture is transferred into a previously cooled autoclave and shaken for 16 hours at room temperature. The autoclave is subsequently cooled to −50°C, opened and freed from liquid ammonia with the simultaneous dropwise addition of n-hexane by evaporation. Thereafter, 100 g of ice and 20 g of glacial acetic acid are added to the mixture, and the n-hexane phase is washed with water, 5-N aqueous sodium hydrogen carbonate solution and again with water, dried over sodium sulphate and evaporated under reduced pressure. The residual [3R]-3-hydroxy-ethynyl-β-ionol, of formula



(XVII),

is reacted further as follows:

12 g of [3R]-3-hydroxy-ethynyl-β-ionol are dissolved in 30 ml of n-hexane. The solution is hydrogenated while stirring at 20°C after the addition of 300 mg of Lindlar catalyst, 180 mg of 2-dimethylaminoethanol and 3 mg of 1,2-bis(2-hydroxy-ethylthio) ethane. After the termination of the hydrogenation, the catalyst is filtered off and the solvent is evaporated under reduced pressure. The residual [3R]-3-hydroxy-vinyl-β-ionol, of formula



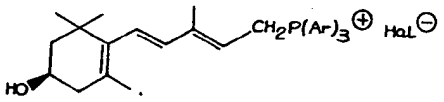
(XVIII),

can be purified by adsorption on aluminium oxide (activity grade III; eluent: ether).

The [3R]-3-hydroxy-vinyl-β-ionol can also be prepared as follows:

A solution of 22.7 g of [3R]-hydroxy-β-ionone in 150 ml of absolute toluene is added dropwise to a solution of 28.4 g of vinyl-magnesium chloride in 114 ml of absolute tetrahydrofuran and 200 ml of absolute toluene. The mixture is subsequently stirred for 1 hour at room temperature, then cooled to 0°—5°C, treated with 0.6—N aqueous ammonium hydroxide solution and saturated aqueous ammonium chloride solution and extracted with ether. The ether extract is washed neutral with saturated aqueous sodium chloride solution, dried and evaporated to dryness. The residual oily [3R]-3-hydroxy-vinyl-β-ionol can be purified by absorption on aluminium oxide (activity grade IV; eluent: ether) and is reacted further as follows:—

15.4 g of [3R]-3-hydroxy-vinyl-β-ionol are dissolved in 300 ml of absolute methanol. After the addition of 17.1 g of triphenyl phosphine, 26 mg of 2,6-di(t-butyl)-p-cresol and 8.5 ml of 25% aqueous hydrochloric acid, the solution is stirred for 18 hours at room temperature. The solvent is subsequently evaporated off under reduced pressure at 40°C and the residue is crystallised from hot acetone. The precipitated [3R]-3-hydroxy-β-ionylidine-ethyl-triphenyl-phosphonium chloride of formula



(XIX),

wherein Ar is phenyl and Hal is chlorine, melts at 211°—212°C after recrystallisation from methylene chloride/acetone/ethyl acetate; $[\alpha]_D^{25}=-57.2°$ (c=1 in chloroform).

1.291 g of [3R]-3-hydroxy-β-ionylidene-ethyl-triphenyl-phosphonium chloride and 162 mg of 2,7-dimethyl-octa-2,6-dien-4-yn-1,10-dial (C_{10}-dialdehyde) are dissolved in 20 ml of methylene chloride. To the resulting homogeneous solution is added 0.364 ml of a 38% aqueous potassium hydroxide solution at −10°C to −14°C with stirring. The mixture is stirred for 1 hour at −10°C to −14°C and subsequently diluted with methylene chloride. The methylene chloride phase is washed neutral with water, dried over sodium sulphate and evaporated under reduced pressure. The residual crude cis/trans-[3R,3'R]-15,15'-didehydrozeaxanthin is crystallised by trituration with 6 ml of warm 90% aqueous methanol. The crystal suspension obtained is cooled to −18°C, the [3R,3'R]-15,15'-didehydrozeaxanthin filtered off, dried and subsequently isomerised as follows:

477 mg of cis/trans-[3R,3'R]-15,15'-didehydrozeaxanthin are dispersed in 5 ml of n-heptane, treated with 5 drops of a 0.1% solution of iodine in chloroform and heated for 18 hours at 90°C while stirring. Subsequently, the n-heptane is evaporated off under reduced pressure. The residual all-trans [3R,3'R]-15,15'-didehydrozeaxanthin melts at 210°—212°C after recrystallisation from methylene chloride/n-hexane.

The all-trans-[3R,3'R]-15,15'-didehydrozeaxanthin can be converted into all-trans-[3R,3'R]-zeaxanthin in accordance with Example 11.

Example 14.

If, in the procedure described in Example 13, the 2,7-dimethyl-octa-2,6-dien-4-yn-1,10-dial is replaced by 2,7-dimethyl-2,4,6-trien-1,10-dial, there is obtained, after condensation with [3R]-3-hydroxy-β-ionylidene-ethyl-triphenyl-phosphonium chloride and after isomerisation of the resulting cis/trans-[3R,3'R]-zeaxanthin, the desired all-trans-[3R,3'R]-zeaxanthin which melts at 208—209°C after recrystallisation from methylene chloride/n-hexane.

Example 15.

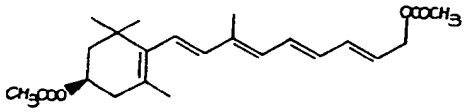
The [3R]-3-hydroxy-β-ionylidene-ethyl-triphenylphosphonium chloride used in Examples 13 and 14 can be replaced by [3R]-3-hydroxy-β-ionylidene-ethyl-triphenyl-phosphonium bromide which can be prepared as follows:

1.6 g of [3R]-3-hydroxy-vinyl-β-ionyl are dissolved in 30 ml of absolute methanol. After the addition of 2.33 g of triphenyl-phosphine hydrobromide, the solution is stirred for 18 hours at room temperature. The solvent is subsequently

evaporated off under reduced pressure and the residue crystallised from hot acetone. The [3R]-3-hydroxy-β-ionylidene-ethyltriphenyl-phosphonium bromide (XIX, Ar = phenyl, Hal = bromine) obtained melts at 186°—187°C after recrystallisation from acetone; $[\alpha]_D^{25}=-55.1°$ (c=1 in chloroform).

Example 16.

5.16 g of [3R]-3-hydroxy-β-ionylidene-ethyl-triphenylphosphonium chloride and 1.49 of γ-acetoxy-tiglic aldehyde are dissolved in 120 ml of methylene chloride. To this solution is added dropwise a solution of 1.30 g of 86% potassium hydroxide in 1.65 ml of water at −35°C while stirring. The mixture is stirred for 1 hour at −35°C and subsequently diluted with cold methylene chloride. The methylene chloride phase is washed neutral with cold saturated aqueous sodium chloride solution, dried and evaporated under reduced pressure. The residue is dissolved in n-hexane and extracted with 60% aqueous methanol. The hexane phase is dried and evaporated under reduced pressure. The residual [3R]-3-hydroxy-retinyl acetate (which consists of about 46% all-trans- and about 48% 11-cis-[3R]-3-hydroxy-retinyl acetate) or the corresponding [3R]-3-acetoxy-retinyl acetate of formula

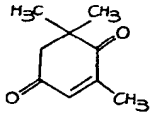


(XX)

obtained therefrom by reaction with acetic anhydride in pyridine can be isomerised according to Example 10 in order to increase the all-trans portion of the product. The all-trans product obtained can subsequently be converted into [3R,3'R]-zeaxanthin according to Example 10. The saponification of the 3-acetoxy group is carried out on the [3R,3'R]-O-acetyl-zeaxanthin obtained by stirring with 1-N aqueous sodium hydroxide solution and methylene chloride at 50°—60°C.

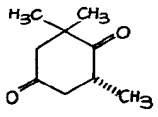
WHAT WE CLAIM IS:—

1. A process for the manufacture of optically active cyclohexane derivatives, which process comprises fermentatively hydrogenating ketoisophorone of the formula



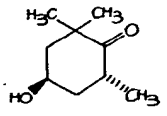
(I)

in an aqueous medium, isolating the resulting [6R]-2,2,6-trimethyl-1,4-cyclohexanedione of the formula



(II)

from the fermentation broth, subjecting said [6R]-2,2,6-trimethyl-1,4-cyclohexanedione to a reduction and, if desired, converting the resulting [4R,6R]-4-hydroxy-2,2,6-trimethylcyclohexanone of the formula



(III)

into an optically active carotenoid.

2. A process according to claim 1, wherein the fermentative hydrogenation is carried out under aerobic conditions.

3. A process according to claim 1 or claim 2, wherein the fermentative hydrogenation is carried out in the presence of a source of assimilable carbon.

4. A process according to claim 3, wherein there is used as the source of assimilable carbon a sugar in an amount of 10—100 g per litre of aqueous medium.

5. A process according to any one of claims 1 to 4 inclusive, wherein the fermentative hydrogenation is carried out in the presence of a source of assimilable nitrogen.

6. A process according to claim 5, wherein there is used as the source of assimilable nitrogen yeast extract in an amount of 1—50 g per litre of aqueous medium.

7. A process according to any one of claims 1 to 6 inclusive, wherein the fermentative hydrogenation is carried out at a pH of 2 to 10.

8. A process according to claim 7, wherein the fermentative hydrogenation is carried out at a pH of 3 to 8.

9. A process according to any one of claims 1 to 8 inclusive, wherein the fermentative hydrogenation is carried out at a temperature of 15°C to 35°C.

10. A process according to any one of claims 1 to 8 inclusive, wherein the fermentative hydrogenation is carried out at a temperature of 25°C to 35°C.

11. A process according to any one of claims 1 to 10 inclusive, wherein there is used a single addition of ketoisophorone in a concentration of 0.1—2.0% based on the aqueous medium.

12. A process according to any one of claims 1 to 10 inclusive, wherein there is used a single addition of ketoisophorone in a concentration of 0.5—1.5% based on the aqueous medium.

13. A process according to any one of claims 1 to 12 inclusive, wherein the fermentative hydrogenation is carried out using Saccharomyces cerevisiae (press-yeast; bakers' yeast) as the microorganism.

14. A process according to claim 13, wherein, after an initial addition of ketoisophorone in a concentration of 0.1—2.0% and subsequent fermentative hydrogenation, the total concentration of ketoisophorone reacted is increased up to 10% by the repeated addition of ketoisophorone in a concentraion of 0.5—1% and subsequent fermentative hydrogenation.

15. A process according to claim 14, wherein there is used a first addition of ketoisophorone in a concentration of 0.5—1.5% and wherein subsequent ketoisophorone additions are repeated until a total concentration of ketoisophorone reacted amounts to 6—8%.

16. A process according to claim 14 or claim 15, wherein the fermentative hydrogenation is carried out at a temperature of 15°C to 25°C.

17. A process according to any one of claims 1 to 12 inclusive, wherein the fermentative hydrogenation is carried out using a microorganism from the genus

Candida
Kloeckera
Rhodotorula
Saccharomyces
Torula
Torulopsis
Aspergillus
Cunninghamella
Curvularia
Cylindrocarpon
Fusarium
Hypomyces
Mucor
Neurospora
Penicillium
Rhizopus
Trichothecium
Arthrobacter (Corynebacterium)
Bacillus
Lactobacillus
Micrococcus
Propionibacterium
Pediococcus

Staphylococcus
Streptococcus
Sarcina
Acetobacter
Acetomonas
Aerobacter
Alcaligenes
Azotobacter
Escherichia
Flavobacter
Klebsiella
Pseudomonas
Proteus
Salmonella
Serratia
Vibrio
Actinomyces
Mycobacterium
Nocardia
Streptomyces and
Proactinomyces.

18. A process according to any one of the claims 1 to 12 inclusive, wherein the fermentative hydrogenation is carried out using a microbially infected soil sample or water sample.

19. A process according to any one of claims 1 to 18 inclusive, wherein the [6R]-2,2,6-trimethyl-1,4-cyclohexanedione of formula II is isolated by filtering or centrifuging the aqueous medium, separating the aqueous phase and the sediment, extracting the separated aqueous phase and the separated sediment with a water-insoluble solvent and isolating the desired product from the solvent extract.

20. A process according to claim 19, wherein methylene chloride is used as the water-insoluble solvent.

21. A process according to any one of claims 1 to 20 inclusive, wherein the reduction of the [6R]-2,2,6-trimethyl-1,4-cyclohexanedione of formula II is carried out using a β-branched aluminium tri(lower alkyl) or a corresponding halo-substituted derivative thereof.

22. A process according to claim 21, wherein triisobutyl-aluminium is used as the β-branched aluminium tri(lower alkyl).

23. A process according to claim 21, wherein isobutyl-aluminium dichloride is used as the halo-substituted derivative of a β-branched aluminium tri(lower alkyl).

24. A process according to any one of claims 1 to 23 inclusive, wherein the reducing agent and the [6R]-2,2,6-trimethyl-1,4-cyclohexanedione of formula II are used in approximately equimolar amounts.

25. A process according to any one of claims 21 to 24 inclusive, wherein the reduction of the [6R]-2,2,6-trimethyl-1,4-cyclohexanedione of formula II is carried out in an inert organic solvent at a temperature between about −70°C and room temperature.

26. A process according to claim 25, wherein said solvent is methylene chloride or a mixture of n-hexane and benzene.

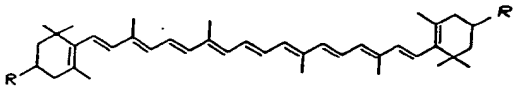
27. A process according to any one of claims 1 to 20 inclusive, wherein the reduction of the [6R]-2,2,6-trimethyl-1,4-cyclohexanedione of formula II is carried out by catalytic hydrogenation using Raney-nickel as the catalyst.

28. A process according to claim 27, wherein the catalytic hydrogenation is carried out in an inert organic solvent at a temperature between about 0°C and about 50°C.

29. A process according to claim 28, wherein the catalytic hydrogenation is carried out in a lower alkanol containing an approximately 5—20% addition of glacial acetic acid and at room temperature.

30. A process according to claim 29, wherein said lower alkanol is methanol.

31. A process according to any one of claims 1 to 30 inclusive, wherein the [4R,6R]-4-hydroxy-2,2,6-trimethyl-cyclohexanone of formula III is converted by chain-lengthening reactions which are known per se in carotenoid chemistry into [3R]-β-cryptoxanthin or [3R,3'R]-zeaxanthin or derivatives thereof of the general formula



(IV),

wherein the R-substituents represent hydrogen, a hydroxy group having the R-configuration or an ether or ester group convertible by hydrolysis into a hydroxy group having the R-configuration, subject to the proviso that at least one of the R-substitutents represents other than hydrogen, and ether or ester groups present are hydrolysed.

32. A process according to claim 31, wherin the [4R,6R]-4-hydroxy-2,2,6-trimethyl-cyclohexanone of formula III is reacted with but-3-yn-2-ol, the resulting 2-hydroxy-4-[[4R,6R]-1,4-dihydroxy-2,2,6-trimethyl-cyclohex-1-yl]-but-3-yne of formula VIII hereinbefore is acetylated to give 2-acetoxy-4-[[4R,6R]-4-acetoxy-1-hydroxy-2,2,6-trimethyl-cyclohex-1-yl]-but-3-yne of formula IX (hereinbefore) said diacetate of formula IX is dehydrated to give 2-acetoxy-4-[[4R]-4-acetoxy-2,6,6-trimethyl-cyclohex-1-en-1-yl]-but-3-yne of formula X (hereinbefore) and the acetylenic bond present is hydrogenated to an ethylenic bond, the resulting [3R]-3-hydroxy-β-ionol of formula XI (hereinbefore) is converted by reaction with a triarylphosphonium halide or with a triaryl phosphine in the presence of a mineral acid into a 4-[[4R]-4-hydroxy-2,2,6-trimethyl-cyclohex-1-en-1-yl]-but-3-ene-2-triarylphosphonium halide of formula XII (hereinbefore) and this Wittig salt is condensed with 1-acetoxy-3-methyl-hexa-2,4-dien-6-al to give [3R]-3-hydroxyretinyl acetate of formula XIII (hereinbefore), the cis form obtained is converted into the trans form by isomerisation, the one half of the [3R]-3-hydroxy-retinyl acetate of formula XIII is converted by reaction with a triarylphosphonium halide or with a triaryl phosphine in the presence of a mineral acid into a [3R]-3-hydroxy-retinyl-triarylphosphonium halide of formula XIV (hereinbefore) and the other half of the [3R]-3-hydroxy-retinyl acetate of formula XIII is saponified to give [3R]-3-hydroxy-retinol of formula XV (hereinbefore) and this alcohol is oxidised to give [3R]-3-hydroxy-retinal of formula XVI (hereinbefore); whereafter said [3R]-3-hydroxy-retinyl-triarylphosphonium halide of formula XIV is either condensed with retinal or with [3R]-3-hydroxy-retinal of formula XVI to give [3R]-β-cryptoxanthin or [3R,3'R]-zeaxanthin.

33. A process according to claim 31, wherein the [4R,6R]-4-hydroxy-2,2,6-trimethyl-cyclohexanone of formula III is reacted with but-3-yn-2-ol, the resulting 2-hydroxy-[[4R,6R]-1,4-dihydroxy-2,2,6-trimethyl-cyclohex-1-yl]-but-3-yne of formula VIII (hereinbefore) is acetylated to give 2-acetoxy-4-[[4R,6R]-4-acetoxy-1-hydroxy-2,2,6-trimethyl-cyclohex-1-yl]-but-3-yne of formula IX (hereinbefore), said diacetate of formula IX is dehydrated to give 2-acetoxy-4-[[4R]-4-acetoxy-2,6,6-trimethyl-cyclohex-1-en-1-yl]-but-3-yne of formula X (hereinbefore) and the acetylenic bond present is hydrogenated to an ethylenic bond, the resulting [3R]-3-hydroxy-β-ionol of formula XI (hereinbefore) is converted by reaction with a triarylphosphonium halide or with a triaryl phosphine in the presence of a mineral acid into a 4-[[4R]-4-hydroxy-2,6,6-trimethyl-cyclohex-1-en-1-yl]-but-3-ene-2-triarylphosphonium halide of formula XII (hereinbefore), this Wittig salt of formula XII is condensed with 4,9-dimethyldodeca-2,4,8,10-tetraen-6-yne-1,12-dial and the resulting [3R,3'R]-15,15'-didehydro-zeaxanthin, after isomerisation of cis form obtained to trans form, is converted by partial hydrogenation into [3R,3'R]-zeaxanthin.

34. A process according to claim 31, wherein the [4R,6R]-4-hydroxy-2,2,6-trimethyl-cyclohexanone of formula III is reacted with but-3-yn-2-ol, the resulting 2-hydroxy-[[4R,6R]-1,4-dihydroxy-2,2,6-trimethyl-cyclohex-1-yl]-but-3-yne of formula VIII (hereinbefore) is acetylated to give 2-acetoxy-4-[[4R,6R]-4-acetoxy-1-hydroxy-2,2,6-trimethylcyclohex-1-yl]-but-3-yne of formula IX (hereinbefore), said diacetate of formula IX is dehydrated to give 2-acetoxy-4-[[4R]-4-acetoxy-2,6,6-trimethyl-cyclohex-1-en-1-yl]-but-3-yne of formula X (hereinbefore) and the acetylenic bond present is hydrogenated to an ethylenic bond, the resulting [3R]-3-hydroxy-β-ionol of formula XI (hereinbefore) is converted by reaction with a triarylphosphonium halide or with a triaryl phosphine in the presence of a mineral acid into a 4-[[4R]-4-hydroxy-2,6,6-trimethyl-cyclohex-1-en-1-yl]-but-3-ene-2-triarylphosphonium halide of formula XII (hereinbefore) and this Wittig salt of formula XII is condensed with 4,9-dimethyl-dodeca-2,4,6,8,10-pentaene-1,12-dial to give [3R,3'R]-zeaxanthin and cis form obtained is converted into trans form by isomerisation.

35. A process according to claim 31, wherein the [4R,6R]-4-hydroxy-2,2,6-trimethyl-cyclohexanone of formula III is reacted with but-3-yn-2-ol, the resulting 2-hydroxy-[[4R,6R]-1,4-dihydroxy-2,2,6-trimethyl-cyclohex-1-yl]-but-3-yne of formula VIII (hereinbefore) is acetylated to give 2-acetoxy-4-[[4R,6R]-4-acetoxy-1-hydroxy-2,2,6-trimethyl-cyclohex-1-yl]-but-3-yne of formula IX (hereinbefore),

said diacetate of formula IX is dehydrated to give 2-acetoxy-4-[[4R]-4-acetoxy-2,6,6-trimethyl-cyclohex-1-en-1-yl]-but-3-yne of formula X (hereinbefore) and the acetylenic bond present is hydrogenated to the ethylenic bond, the resulting [3R]-3-hydroxy-β-ionol of formula XI (hereinbefore) is converted by oxidation into [3R]-3-hydroxy-β-ionone, said ionone is reacted with an alkali metal acetylide to give [3R]-3-hydroxy-ethynyl-β-ionol of formula XVII (hereinbefore), this latter compound is catalytically hydrogenated and the resulting [3R]-3-hydroxyvinyl-β-ionol of formula XVIII (hereinbefore) is converted by reaction with a triarylphosphonium halide or with a triarylphosphine in the presence of a mineral acid into a [3R]-3-hydroxy-β-ionylidenethyl-triaryl-phosphonium halide of formula XIX (hereinbefore), said phosphonium halide is condensed with 2,7-dimethyl-octa-2,4-dien-4-yne-1,10-dial and the resulting [3R,3'R]-15,15'-didehydro-zeaxanthin, after isomerisation of cis form obtained to trans form, is converted by partial hydrogenation into [3R,3'R]-zeaxanthin.

36. A process according to claim 31, wherein the [4R,6R]-4-hydroxy-2,2,6-trimethyl-cyclohexanone of formula III is reacted with but-3-yn-2-ol, the resulting 2-hydroxy[[4R,6R]-1,4-dihydroxy-2,2,6-trimethyl-cyclohex-1-yl]-but-3-yne of formula VIII (hereinbefore) is acetylated to give 2-acetoxy-4-[[4R,6R]-4-acetoxy-1-hydroxy-2,2,6-trimethyl-cyclohex-1-yl]-but-3-yne of formula IX (hereinbefore), said diacetate of formula IX is dehydrated to give 2-acetoxy-4-[[4R]-4-acetoxy-2,6,6-trimethyl-cyclohex-1-en-1-yl]-but-3-yne of formula X (hereinbefore) and the acetylenic bond present is hydrogenated to an ethylenic bond, the resulting [3R]-3-hydroxy-β-ionol of formula XI (hereinbefore) is converted by oxidation into [3R]-3-hydroxy-β-ionone, said ionone is reacted with an alkali metal acetylide to give [3R]-3-hydroxy-ethynyl-β-ionol of formula XVII (hereinbefore), this latter compound is catalytically hydrogenated and the resulting [3R]-3-hydroxyvinyl-β-ionol of formula XVIII (hereinbefore) is converted by reaction with a triarylphosphonium halide or with a triarylphosphine in the presence of a mineral acid into a [3R]-3-hydroxy-β-ionylidenethyl-triaryl-phosphonium halide of formula XIX (hereinbefore), said phosphonium halide is condensed with 2,7-dimethyl-octa-2,4,6-triene-1,10-dial to give [3R,3'R]-zeaxanthin and cis form obtained is converted into trans form by isomerisation.

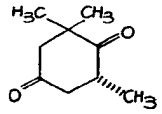
37. A process according to claim 31, wherein the [4R,6R]-4-hydroxy-2,2,6-trimethyl-cyclohexanone of formula III is reacted with but-3-yn-2-ol, the resulting 2-hydroxy-[[4R,6R]-1,4-dihydroxy-2,2,6-trimethyl-cyclohex-1-yl]-but-3-yne of formula VIII (hereinbefore) is acetylated to give 2-acetoxy-4-[[4R,6R]-4-acetoxy-1-hydroxy-2,2,6-trimethyl-cyclohex-1-yl]-but-3-yne of formula IX (hereinbefore), said diacetate is dehydrated to give 2-acetoxy-4-[[4R]-4-acetoxy-2,6,6-trimethyl-cyclohex-1-en-1-yl]-but-3-yne of formula X (hereinbefore) and the ethylenic bond present is hydrogenated to an ethylenic bond, the resulting [3R]-3-hydroxy-β-ionol of formula XI (hereinbefore) is converted by oxidation into [3R]-3-hydroxy-β-ionone, said ionone is reacted with an alkali metal acetylide to give [3R]-3-hydroxy-ethynyl-β-ionol of formula XVII (hereinbefore), this latter compound is catalytically hydrogenated and the resulting [3R]-3-hydroxy-vinyl-β-ionol of formula XVIII (hereinbefore) is reacted with a triarylphosphonium halide or with a triarylphosphine in the presence of a mineral acid to give a [3R]-3-hydroxy-β-ionylidenethyl-triaryl-phosphonium halide of formula XIX (hereinbefore), said phosphonium halide is reacted with p-acetoxytiglic aldehyde to give [3R]-3-hydroxy-retinyl acetate of formula XIII (hereinbefore), cis form obtained is converted into trans form by isomerisation, the one half of the [3R]-3-hydroxy-retinyl acetate of formula XIII is converted by reaction with a triarylphosphonium halide or with a triarylphosphine in the presence of a mineral acid into a [3R]-3-hydroxy-retinyl-triarylphosphonium halide of formula XIV (hereinbefore) and the other half of the [3R]-3-hydroxy-retinyl acetate of formula XIII is saponified to give [3R]-3-hydroxy-retinol of formula XV (hereinbefore) and this alcohol is oxidised to give [3R]-3-hydroxy-retinyl of formula XVI (hereinbefore); whereafter said [3R]-3-hydroxy-retinyl-triarylphosphonium halide of formula XIV is either condensed with retinal or with [3R]-3-hydroxy-retinal of formula XVI to give [3R]-β-cryptoxanthin or [3R,3'R]-zeaxanthin.

38. A process according to claim 31, wherein the [4R,6R]-4-hydroxy-2,2,6-trimethyl-cyclohexanone of formula III is reacted with but-3-yn-2-ol, the resulting 2-hydroxy-[[4R,6R]-1,4-dihydroxy-2,2,6-trimethyl-cyclohex-1-yl]-but-3-yne of formula VIII (hereinbefore) is acetylated to give 2-acetoxy-4-[[4R,6R]-4-acetoxy-1-hydroxy-2,2,6-trimethyl-cyclohex-1-yl]-but-3-yne of formula IX (hereinbefore), said diacetate of formula IX is dehydrated to give 2-acetoxy-

4-[[4R]-4-acetoxy-2,6,6-trimethyl-cyclohex-1-en-1-yl]-but-3-yne of formula X (hereinbefore) and the acetylenic bond present is hydrogenated to an ethylenic bond, the resulting [3R]-3-hydroxy-β-ionol of formula XI (hereinbefore) is converted by oxidation into [3R]-3-hydroxy-β-ionone, said ionone is reacted with an alkali metal acetylide to give [3R]-3-hydroxy-ethynl-β-ionol of formula XVII hereinbefore, this latter compound is catalytically hydrogenated and the resulting [3R]-3-hydroxy-vinyl-β-ionol of formula XVIII hereinbefore is converted by reaction with a triarylphosphonium halide or with a triarylphosphine in the presence of a mineral acid into a [3R]-3-hydroxy-β-ionylidenethyl-triarylphosphonium halide of formula XIX hereinbefore, said phosphonium halide is reacted with γ-acetoxy-tiglic aldehyde to give [3R]-3-hydroxy-retinyl acetate of formula XIII hereinbefore, said acetate of formula XIII is acetylated to give [3R]-3-acetoxy-retinyl acetate of formula XX hereinbefore, cis form obtained is converted into trans form by isomerisation, the one half of the [3R]-3-acetoxy-retinyl acetate of formula XX is converted by reaction with a triarylphosphonium halide or with triarylphosphine in the presence of a mineral acid into a [3R]-3-acetoxy-retinyl-triarylphosphonium halide of formula XXI hereinbefore and the other half of the [3R]-3-acetoxy-retinyl acetate of formula XX is saponified to give [3R]-3-hydroxy-retinol of formula XV hereinbefore and this alcohol is oxidised to give [3R]-3-hydroxy-retinal of formula XVI hereinbefore whereafter said [3R]-3-acetoxy-retinyl-triarylphosphonium halide of formula XXI is reacted either with retinal or with [3R]-3-hydroxy-retinal of formula XVI to give [3R]-O-acetyl-β-cryptoxanthin or [3R,3'R]-O-acetyl-zeaxanthin respectively and the latter compounds are saponified to give [3R]-β-cryptoxanthin or [3R,3'R]-zeaxanthin respectively.

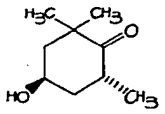
39. A process as claimed in any of claims 32 to 38 wherein, before reaction with but-3-yn-2-ol, the free hydroxy group of the compound of formula III is masked by treatment with isopropenylmethyl ether.

40. [6R]-2,2,6-Trimethyl-1,4-cyclohexanedione of the formula



(II)

41. [4R,6R]-4-Hydroxy-2,2,6-trimethyl-cyclohexanone of the formula



(III)

42. A process as claimed in claim 1 for the manufacture of optically active cyclohexane derivatives, substantially as hereinbefore described with reference to the foregoing Examples.

43. An optically active cyclohexane derivative, when manufactured by the process claimed in any one of claims 1 to 39 inclusive of claim 42.

For the Applicants,
CARPMAELS & RANSFORD,
Chartered Patent Agents,
43 Bloomsbury Square,
London WC1A 2RA.

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